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【 CASE REPORT 】

Isolated Pancreatic Myeloid Sarcoma Associated with t(8;21)/*RUNX1-RUNX1T1* Rearrangement

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Abstract:
No valid treatment for isolated myeloid sarcoma (IMS) has yet been established, and no thorough genetic examinations have been performed because of its low incidence and unique manner of development. We herein report a 34-year-old man with pancreatic IMS with t(8;21)/*RUNX1-RUNX1T1* rearrangement. He was treated with high-dose cytarabine followed by allogeneic hematopoietic stem cell transplantation (allo-HSCT). This is the first report of pancreatic IMS with t(8;21). Positron emission tomography/computed tomography and genetic study are useful for the diagnosis, and allo-HSCT achieved complete remission in this patient.

Key words: isolated myeloid sarcoma, t(8;21), pancreas

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Introduction

Myeloid sarcoma (MS) is a characteristic disease entity in myeloid neoplasms. In most MS cases, leukemic blasts in the peripheral blood and bone marrow (BM) are found at the diagnosis (1). MS is recognized in approximately 5% of acute myeloid leukemia (AML) cases (2). However, AML cells are not detected in the peripheral blood or BM of isolated MS (IMS) cases. Given that IMS is found in 25% of MS cases (1), IMS is assumed to be present in 1% of AML cases.

MS, including IMS, consists of immature myeloid cells and may develop in lesions throughout the body. The skin, lymph node, testis and digestive tract are common sites of MS. IMS, which is not concomitant with leukemic blasts in the peripheral blood or BM, often lacks specific symptoms and is therefore difficult to diagnose properly. In many cases of IMS, it is difficult to access and obtain sufficient specimens for a diagnosis because of the anatomical sites of the tumors. Occasionally, a needle biopsy is clinically useful. However, needle biopsy samples are not adequate for immunochemistry and genetic analyses.

The low incidence, varied and non-specific clinical symptoms, difficulty in obtaining diagnostic specimens and variety of histological appearance of IMS make its diagnosis and treatment challenging (2, 3). No valid treatment has been established, and how to treat the disease is a matter of concern in clinical hematology.

Case Report

A 34-year-old man complained of a 3-month history of upper abdominal pain. Abdominal echography and computed tomography (CT) images revealed a tumor in the pancreas. Thereafter, he was referred to our hospital (Kumamoto University Hospital, Kumamoto, Japan). The peripheral blood cell counts were all within normal ranges: white blood cell count 4.4×10^9/L, hemoglobin 133 g/L and platelets 2.37×10^{11}/L with no evidence of leukemic blasts in the peripheral blood. Serum lactate dehydrogenase and C-reactive protein levels were elevated at 218 U/L (upper limit of normal, 213) and 10.7 mg/L (upper limit of normal, 3.0), respectively, although levels of other serum liver enzymes, amylase and

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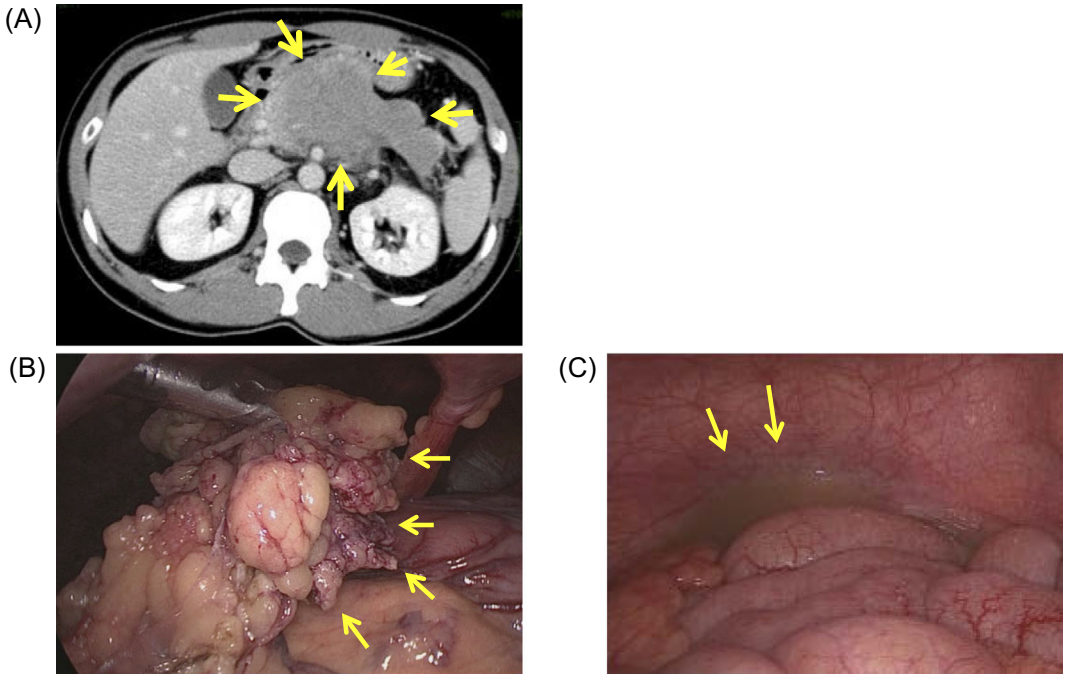

Figure 1. Bulky tumor of the pancreas found with enhanced-contrast computed tomography (CT) and laparoscopy. (A) Contrast-enhanced CT revealed ischemic bulky tumor of the pancreas (arrows). (B) A laparoscopic examination revealed a pancreatic tumor and thick omentum (arrows). (C) Turbid ascites (arrows).

creatinine were not markedly increased. Contrast-enhanced CT showed a 10×6-cm ischemic bulky tumor in the pancreas body and tail with a thickened greater omentum (Fig. 1A).

A tumor biopsy was performed with laparoscopy, and a few milliliters of turbid ascites was obtained (Fig. 1B and C). Cytologically, the tumor cells were immature leukemic blast-like cells with blue-gray cytoplasm and an indented nucleus with fine chromatin without adepithelial connections (Fig. 2, May-Giemsa staining). A histopathological study revealed that the tumor cells were negative for lymphoid and epithelial markers, CD3, CD7, CD20, terminal deoxynucleotidyl transferase (TdT) and cytokeratin AE1/AE3. A flow cytometric analysis and immunohistochemical study revealed that the cells in ascites were positive for myeloid and monocyte antigens, CD4, CD15, CD33, CD34, CD56, CD68/Kp-1 and myeloperoxidase (MPO) (Fig. 2). Therefore, the pancreatic tumor was diagnosed as MS. Positron emission tomography (PET)/CT showed that ^{18}F-fluorodeoxyglucose (FDG) was taken up only by the pancreatic tumor and thickened greater omentum (maximum standard uptake value [SUVmax]: 8.8). Leukemic blasts were not found in the BM by histological or cytological analyses. The patient was diagnosed with pancreatic IMS with invasion to the greater omemtum and ascites. He was treated with the AML regimen of idarubicin for three days and cytarabine for seven days.

The WT1 mRNA level in the peripheral blood increased to 1,000 copies/μgRNA at the diagnosis. The tumor cells obtained by a biopsy were not enough for a G-banding analysis. To predict the prognosis, fluorescence *in situ* hybridization (FISH) for *RUNX1-RUNX1T1* rearrangement was additionally ordered based on the knowledge that such a rearrangement is sometimes found in MS cases. *RUNX1-RUNX1T1* fusion signals were detected in 17.6% of the tumor cells by FISH using the Vysis LSI RUNX1/RUNX1T1 Dual Color Dual Fusion Probes (Abbott Molecular, Des Plaines, USA). In contrast, the fusion signal was not detected in BM cells at the diagnosis. The patient was ultimately diagnosed with pancreatic IMS with t(8;21)(q22;q22)/*RUNX1-RUNX1T1* rearrangement. cKIT exon 8 and 17 mutations were not found by sequencing cDNA extracted from the tumor cells. Reverse transcription polymerase chain reaction (RT-PCR) revealed that *RUNX1-RUNX1T1* fusion mRNA was detected in the tumor cells and the BM mononuclear cells (Fig. 3). These data suggest the presence of a small number of leukemic cells or non-leukemic cells with the *RUNX1-RUNX1T1* fusion gene in the BM.

The idarubicin and cytarabine regimen was followed by three cycles of high-dose cytarabine regimens. The WT1 mRNA level was reduced to 93 copies/μgRNA. In addition, PET/CT revealed no ^{18}F-FDG-positive lesion in the pancreas, and RT-PCR showed no *RUNX1-RUNX1T1*-positive cells in the BM. Therefore, we concluded that our patient had achieved complete remission (CR). He was treated with allogeneic hematopoietic stem cell transplantation (allo-HSCT) from an human leukocyte antigen (HLA)-matched unrelated donor with busulfan and cyclophosphamide as the conditioning regimen using cyclosporine and short-course

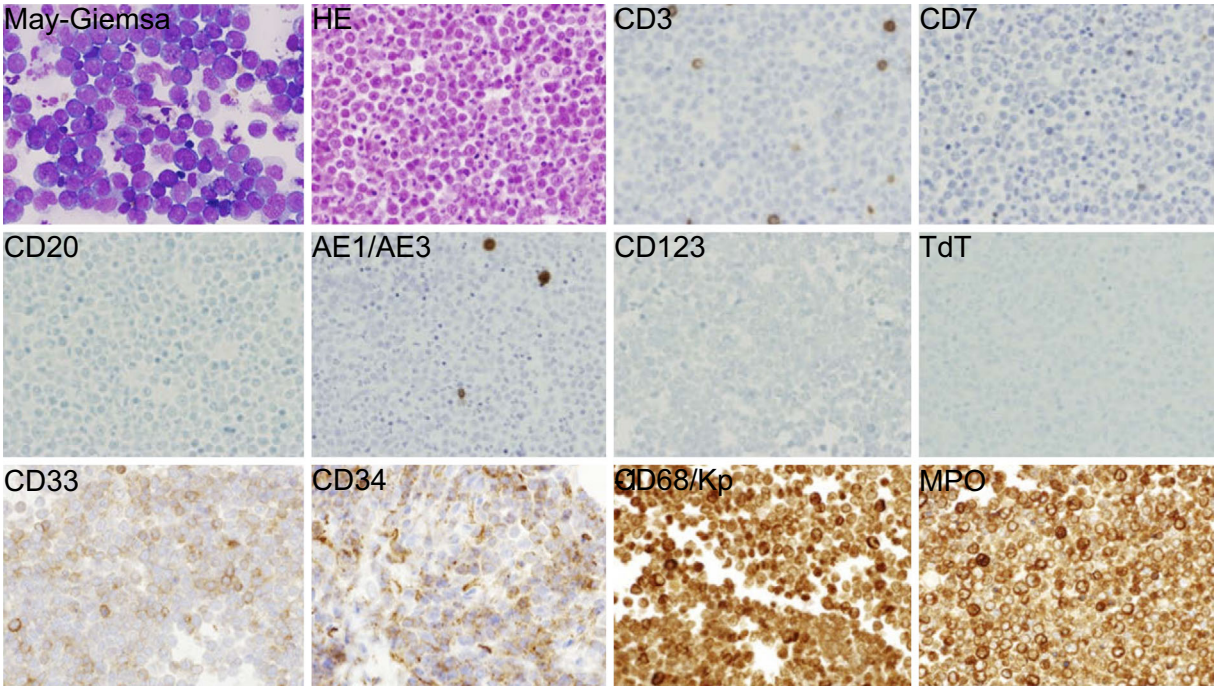

Figure 2. **Cytology and histology of tumor cells. A biopsy of the pancreatic tumor revealed myeloid sarcoma. May-Giemsa staining revealed that the tumor cells with blue-gray cytoplasm were leukemic blast-like cells. An immunohistochemical analysis showed that the tumor cells were negative for lymphoid and epithelial markers, CD3, CD7, CD20, TdT or cytokeratin AE1/AE3, and positive for myeloid and monocyte antigens, CD33, CD34, CD68/Kp-1 and MPO. HE: Hematoxylin and Eosin staining, MPO: myeloperoxidase, TdT: terminal deoxynucleotidyl transferase. Magnification: all photomicrographs ×400**

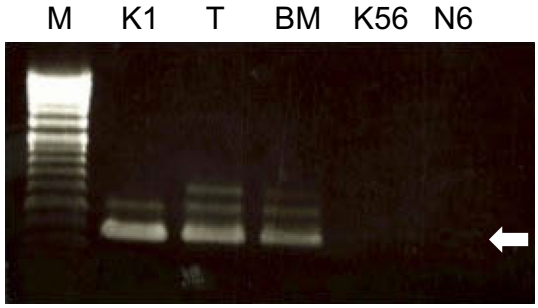

Figure 3. **A genetic analysis with RT-PCR. *RUNX1-RUNX1T1* fusion mRNA was detected by reverse transcription polymerase chain reaction in tumor cells and BM cells at the diagnosis. The Kasumi-1 acute myeloid leukemia cell line was utilized as a positive control representing cells with the *RUNX1-RUNX1T1* gene. The K562 and Nalm6 cell lines were negative controls. The fusion mRNA-specific PCR product length was 395 base pairs (pointed by the white arrow). Larger sized bands are non-specific. BM: bone marrow mononuclear cell, K1: Kasumi1, K56: K562, M: marker, N6: Nalm6, T: tumor cell**

methotrexate as graft-versus-host disease prophylaxis. He has been in CR for 1.5 years since the diagnosis (Fig. 4). The *RUNX1-RUNX1T1* fusion gene has not been detected by PCR, and the WT1 mRNA count has dropped below 50 copies/μgRNA (lower limit of normal) since HSCT.

Discussion

A number of different cytogenetic abnormalities in MS have been reported, but which ones are specific to MS is not known (1, 4). Translocation (8;21) is a common cytogenetic abnormalities. Avni et al. reported that the rate of t(8;21) in MS patients ranged from 3.3% to 43% (5). MS with t(8;21) tends to develop orbitally or peri-orbitally (6). Only 13 cases of pancreatic MS cases, including the present case, have been reported since 1987 (Table) (7-16). Our case is believed to be a rare report of genetically confirmed pancreatic IMS with t(8;21)(q22;q22)/*RUNX1-RUNX1T1* rearrangement. A genetic analysis by FISH is useful for the diagnosis of MS.

In our IMS case, only RT-PCR indicated the presence of cells harboring the *RUNX1-RUNX1T1* gene in BM, which is similar to a previous report showing *RUNX1-RUNX1T1* fusion mRNA in BM in a case of IMS (17). IMS patients subsequently develop AML after an average of 10 months (18, 19). It is possible that there are a small number of leukemic cells in the BM at the diagnosis of IMS, leading to AML. Therefore, IMS should be treated by systemic chemotherapy. There have been no large prospective studies on a suitable therapy for IMS because of its low incidence. The efficacy of cytarabine-based regimens has been reported in small numbers of patients (20-22). In AML with t(8;21),

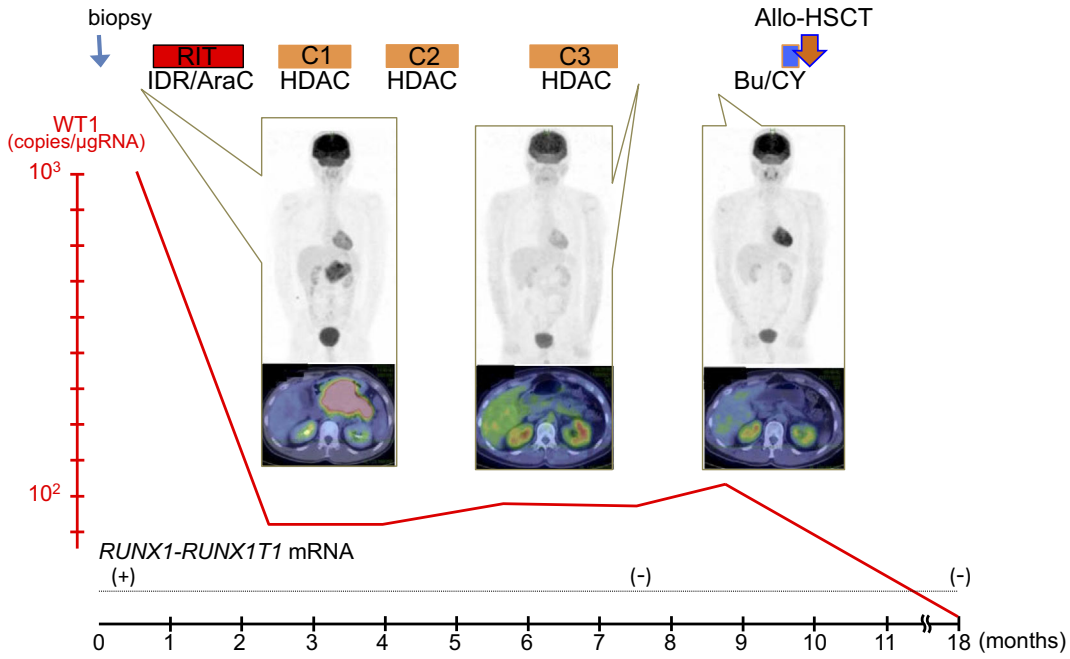

Figure 4. Clinical course of the case of pancreatic isolated myeloid sarcoma. Following chemotherapy, the bulky pancreatic tumor shrank and showed reduced uptake signals on positron emission tomography/computed tomography (PET/CT). WT1 mRNA was reduced to 10^{-1} copies after the consolidative therapies. Allogeneic hematopoietic stem cell transplantation was performed. The conditioning regimen was busulfan and cyclophosphamide. At 18 months after the diagnosis, the WT1 mRNA count dropped below 50 copies/μgRNA (lower limit of normal). *RUNX1-RUNX1T1* mRNA RT-PCR was performed qualitatively. The black bars in the pictures of PET/CT are used to cover identifying patient information. Allo-HSCT: allogeneic hematopoietic stem cell transplantation, Bu/CY: busulfan and cyclophosphamide conditioning, C1-3: consolidation therapy cycles 1-3, HDAC: high dose cytarabine regimen, IDR/AraC: idarubicin and cytarabine regimen, RIT: remission induction therapy, *RUNX1-RUNX1T1* mRNA: results of the qualitative RT-PCR for *RUNX1-RUNX1T1* mRNA, WT1: Wilms' tumor 1 mRNA

MS is assumed to be a poor prognostic factor; the median survival time of patients with AML with t(8;21) is 59.5 months, compared to 5.4 months for patients with AML with t(8;21) and MS (23). Among 12 patients with pancreatic MS, CR of 2 years' duration was achieved in 2 patients treated with chemotherapy combined with surgical resection or HSCT (Cases 2 and 7 in Table). Given the poor prognosis of MS with t(8;21), the previous reports of pancreatic MS, and the existence of disseminative disease, we planned allo-HSCT for our case.

Allo-HSCT in addition to systemic chemotherapy may improve the prognosis of patients with IMS. The retrospective study by Antic et al. revealed that the 5-year overall survival (OS) of 12 IMS patients was 25%, and survival for >50 months was found in 2 of 3 patients treated with HSCT but only 1 of 9 patients without HSCT (24). Chevallier et al. reported that, in 30 patients with IMS who received allo-HSCT, the 5-year OS, leukemia-free survival rate and none-relapse mortality were 33%, 30% and 17%, respectively. In addition, the authors pointed out that having achieved CR at allo-HSCT and lacking a poor prognostic karyotype were indicators of a good prognosis (25). Lazzarotto et al. analyzed the clinical outcome of 48 MS patients, including 9 IMS patients, and similarly reported that allo-HSCT after intensive chemotherapy improved the prognosis (OS probability at 5 years for the whole population and for the 22 MS patients who received allo-HSCT: 33% and 53%, respectively). In addition, having achieved CR at allo-HSCT influenced a better prognosis (26). Allo-HSCT is assumed to improve the prognosis of patients with IMS. Allo-HSCT may be considered the primary treatment option for IMS, although the findings from these retrospective studies may include some selection bias. In addition, the evaluation of the treatment response and cytogenetic information in IMS cases are important.

MS is an extramedullary neoplastic tumor of immature myeloid cells, with various types and numbers of inflammatory cells. There are no known highly sensitive and specific antigens for the diagnosis of MS cells. It is usually difficult to obtain enough specimens for genetic examinations because of the site and size of IMS. However, genetic information is necessary to clarify the origin of IMS and plan treatment. Therefore, we performed a laparoscopic biopsy of the pancreas. Immunophenotyping with a histological analysis, flow cytometry and a genetic analysis of the biopsy samples resulted in a proper diagnosis of IMS.

Table. **Previously Reported Cases of Pancreatic MS.**

No.	Sex/ age	Concomitant AML	Karyotype	Treatment	Clinical course	Reference
1	F/36	No	NA	RT +ChT (CPA+VCR+AraC+PSL)	Relapse as M4Eo with diploidy after 7 months, RIT(DNR+AraC+thoguanine) CR with 7-months follow-up	7
2	M/32	No	NA	Duodenopancreatectomy +ChT (IDR+HDAC) +ChT (amsacrine+ETP)	CR with 2-years follow-up	8
3	F/37	Yes	NA	No	Died 45 days after tumor detection	9
4	M/31	Yes	46, XY	ChT (IDR+AraC+ATRA)	CR (follow-up unknown)	10
5	F/61	Yes	Trisomy 8 and 13	ChT (IDR+AraC)	Relapse after 10 cycles of ChT (IDR+AraC), died	
6	M/64	In CR of M2	NA	ChT (unknown)	CR, died from stroke	11
7	F/42	Yes	47,+mar	ChT (HDAC+IDR) +ChT (IDR+AraC+ETP) +CBT	CR at 49 months after CBT	12
8	F/75	Yes	inv(16)	ChT (ETP+AraC+MIT)	Relapse after 7 months, died	13
9	M/40	No	NA	Duodenopancreatectomy +ChT (AraC)	CR (follow-up unknown)	14
10	F/42	No	NA	Distal pancreatectomy +splenectomy, ChT was refused	Relapse after 2months, died after 3 months	15
11	F/45	No	NA	Duodenopancreatectomy +ChT (CDDP+AraC+DEX)	Early relapse, died	16
12	F/19	No	NA	ChT (AraC-based chemotherapy) +3 cycles of consolidation	Relapse, ChT(amsacrine+AraC) +ChT (AraC) followed by BMT relapse, 6 months after BMT	
13	M/34	No	t(8;21)	ChT (IDR+AraC) +ChT (HDAC, 3 cycles) +allo-BMT	CR with 1.5-year follow-up	Our case

allo-BMT: allogeneic bone marrow transplantation, AraC: cytarabine, ATRA: all-trans retinoic acid, CBT: cord blood transplantation, CDDP: cisplatin, ChT: chemotherapy, CPA: cyclophosphamide, CR: complete remission, DEX: dexamethasone, DNR: daunorubicin, ETP: etoposide, F: female, HDAC: high-dose AraC therapy, IDR: idarubicin, M: male, MIT: mitoxantrone, M2: acute myeloid leukemia M2 by FAB classification, M4Eo: M4 with eosinophilia (FAB), NA: not applicable, PSL: prednisolone, RT: radiotherapy, VCR: vincristine

There is no sensitive method for assessing deep CR, such as flow cytometry or RT-PCR, in IMS. PET/CT is effective for the evaluation of the residual tumor and treatment response. PET/CT can detect MS more sensitively than standard CT and magnetic resonance imaging (27, 28). MS can develop at any site in the body, and PET/CT is useful for screening for MS, finding a biopsy site, and evaluating the treatment response (28, 29). In the present case, ^{18}F-FDG PET/CT were utilized for the assessment of the treatment response.

The authors state that they have no Conflict of Interest (COI).

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